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KING & SPALDING LLP
1180 PEACHTREE STREET
ATLANTA, GA 30309-3521

EXAMINER	
SGAGIAS, MAGDALENE K	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/646,970	PHELPS, CAROL J.	
	Examiner	Art Unit	
	Magdalene K. Sgagias	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 August 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-21,43-46 and 48-64* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-21, 43-46, 48-64* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's arguments filed 8/13/07 have been fully considered but they are not persuasive. The amendment has been entered. Claims 1-21, 43-46, 48-64 are pending and under consideration. Claims 22-42, 47 have been canceled.

Claim Objections

Claims 43 and 48 objection to because claims depend from the cancelled claim 22 is **withdrawn**.

Specification

The disclosure objection to because it contains an embedded hyperlink and/or other form of browser-executable code and because the specification contains inappropriate characters as letters throughout the document is **withdrawn.**

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims **1-16, 40-46** are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter is **withdrawn.**

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims **1-16, 51-59, 61, 63-64** are rejected under 35 U.S.C. 102(a) as being anticipated by **Lai et al,** (Science, 295: 1089-1092, February 2002).

Lai teaches the production of four live pigs in which one allele of the α-1,3-galactosyltransferase locus has been knocked out by nuclear transfer cloning, wherein clonal fetal fibroblasts cell lines were used as nuclear donors for embryos reconstructed for enucleated pigs (abstract and thru ought the document).

Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. See *In re Ludtke* 441 F.2d 660, 169 USPQ 563 (CCPA 1971). Whether the rejection is based on "inherency" under 35 USC 102, or "prima facie obviousness" under 35 USC 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. I*n re Best, Bolton, and Shaw*, 195 USPQ 430, 433 (CCPA 1977) citing *In re Brown*, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972) and In re Fitzgerald, 619 F.2d 67, 70, 205 USPQ 594, 596 (CCPA 1980) (quoting In re Best, 562 F.2d 1252, 1255, 195 USPQ 430, 433-34(CCPA 1977)).

There is no requirement that a person of ordinary skill in the art would have recognized the inherent disclosure at the time of invention, but only that the subject matter is in fact inherent in the prior art reference. Schering Corp. v. Geneva Pharm. Inc., 339 F.3d 1373, 1377, 67 USPQ2d 1664, 1668 (Fed. Cir. 2003). "Products of identical chemical composition can not have mutually exclusive properties." A chemical composition and its properties are inseparable. Therefore, if the prior art teaches the identical chemical structure, the properties applicant discloses and/or claims are necessarily present. In re Spada, 911 F.2d 705, 709, 15 USPQ2d 1655, 1658. Applicant is referred to MPEP 2112 for further discussion on inherency.

Thus, Lai anticipates claimed invention.

Claims **8, 13, 48-50 remain** rejected under 35 U.S.C. 102(b) as being anticipated by **Gustafsson et al,** (6,153,428; Nov 28, 2000).

Gustafsson et al, teaches a tissue of a pig that lacks expression of functional a(1,3) galactosyltransferase (abstract) (claim **8**).

Gustafsson et al, teaches a porcine cell which is interpreted to read on tissue that lacks any expression of functional α(1,3) galactosyltransferase (column 6, lines 2-9) (claims **8** and **13**).

Gustafsson et al, teaches a porcine cell that carries a homozygous knock out for the gal alpha-1,3-GT gene (column 6, lines 1-9 and column 7, lines 1-10). Gustafsson et al, teaches a porcine cell that carries a homozygous knock out for the gal alpha-1,3-GT gene in which at least one allele contains an induced mutation in the α(1,3) galactosyltransferase gene (column 6, lines 40-42). Gustafsson et al, teaches the swine is preferably an alpha(1,3) galactosyltransferase negative swine grown from a porcine oocyte whose pronuclear material has been removed and into which has been introduced a totipotent porcine embryonic stem cell

using protocols for nuclear transfer and ES cells used for nuclear transfer are negative for the expression of alpha.(1,3) galactosyl transferase, or alternatively, totipotent ES cells used for nuclear transfer are mutated in a targeted fashion in at least one allele of the alpha(1,3) galactosyltransferase gene (column 5, lines 41-50) **(claims 48-50).**

The cells of claims 48-50 are the same as those of Gustafsson since the only requirement is knock out alpha-1,3-GT gene in which at least one allele contains an induced mutation in the α(1,3) galactosyltransferase gene (column 6, lines 40-42); column 6, lines 2-9), (claims **48-50**). As such Gustafsson anticipates claimed tissues and cells of the dependent claims **8, 13, 48-50**.

Applicants argue that they disagree that Gustafsson teaches any tissues or cells from a pig that lacks expression of functional alpha(1,3)GT, as recited in the pending claims. Applicants argue that the present invention is based on the first successful birth of viable pigs that lack any expression of functional alpha(1,3)GT. Applicants argue that despite the fact that the production of alpha 1,3 gal null pigs has been a goal in the field of xenotransplantation for decades, as noted in the specification, a homozygous alpha(1,3)GT knock out pig was not produced until 2002, as part of the present invention and which was published in Science in January, 2003 by Phelps et al. (Phelps, et al. Science 299: 411-414, (January 17, 2003).

These arguments are not persuasive because claimed invention is not limited to homozygous alpha(1,3)GT pigs but the breadth of the claimed invention is broader than the teachings of the specification as well as the published version of the invention in Science in January, 2003 which teaches the production of homozygous alpha(1,3)GT deficient pigs by nuclear transfer technology, wherein the specific exon 9 mutation of the second allele is speculated to be either a probability of a natural mutation or an induced mutation in the embryo as cited in said science paper:

"Thus, we have successfully produced four α1,3GT-deficient piglets by a toxin A–mediated selection method. Although our intent was to knock out the second allele of the α1,3GT gene by homologous recombination, this did not occur. Instead, because we used this powerful selection method, which allows us to isolate any event that results in loss of α1,3GT activity, we discovered a mutation in the second allele of the α1,3GT gene. Had we used standard selection methods with puromycin or hygromycin, we would not have found the mutation. Although the rate of spontaneous mutation in the pig genome is very low [about 4 x10_^{8} for a spontaneous mutation per replication (*18*) in a mammalian gene similar in size to the α1,3GT gene], toxin A selection still enabled us to detect this crucial mutation. Clearly inactivation of the α1,3GT protein by this point mutation is a better outcome than by gene targeting with the pPL680 vector. It provides the opportunity to produce α 1,3GT-deficient pigs without any antibiotic-resistance genes or other foreign DNA sequences, which should facilitate regulatory approval and, potentially, make a safer product for human use. It is certain that this point mutation will be maintained in the genome of these DKO pigs and their offspring, just as the few critical point mutations in the α 1,3GT gene of humans and higher primates have been maintained over 20 million years (*14*). This genomic stability is not only due to the rarity of a reverse mutation event [about 5 x10_^{11} per replication (*18*) for mammals] but, more importantly, the strong selection pressure against α 1,3Gal-positive cells by the presence of antibodies to α 1,3Gal in α 1,3Gal-negative animals. Our results have demonstrated that removal of α 1,3Gal epitopes on pig cells did not preclude development in utero, even though pig cells express up to 500 times the number of α 1,3Gal epitopes as do mouse cells (*4*, *19*). In addition, three consecutive rounds of cloning with rederived fetal cells did not appear to have a major detrimental effect on the overall development or health of

the cloned pigs in this study. Analysis of tissues and organs from these α1,3GT DKO pigs in nonhuman primate models should provide clear indications of the involvement of α 1,3Gal in HAR, AVR, and chronic rejection (p 413, 2nd and 3rd column).

Since the only requirement is knock out of alpha-1,3-GT gene in which at least one allele contains an induced mutation in the α(1,3) galactosyltransferase gene that lacks any expression of functional α(1,3) galactosyltransferase, Gustafsson anticipates claimed invention.

Applicants argue that Gustafsson does include prophetic example for making a swine null for a(1,3)GT and Gustafsson suggests using porcine embryonic stem cell cultures to produce the animals yet, unlike mice, embryonic pig stem cells have not been useful to produce transgenic animals.

These arguments are not persuasive because claimed invention is not limited to ES cells and Gustafsson teaches the production of claimed pigs by nuclear transfer technology.

Claims **1-16, 43-44, 46, 48-49** are rejected under 35 U.S.C. 102(e) as being anticipated by **Denning et al,** (US 7,126,039 B2, Date of Patent: Oct. 24, 2006; Filed: Mar. 21, 2002).

Denning et al, teaches a pig that lacks expression of alpha.1,3 GT gene, (see claim 1, and column 99, lines 26-27), (claim **1**)

Denning et al, teaches and organ of a pig that lacks expression of functional of alpha,1,3 GT gene, wherein the organ is kidney, liver, heart, lung, pancreas, (column 22, lines 53-61) (claims **2-7**).

Denning et al, teaches a tissue of a pig that lacks expression of functional alpha 1,3 GT gene, wherein the tissue is any type of tissue such as solid tissue, cartilage (column 22, lines 58-59) (claims **8-12**).

Denning et al, teaches a cell from a pig a pig that lacks expression of functional of alpha,1,3 GT gene, wherein the cell is derived from the pancreas, Langerhans cell or insulin secreting cell (column 22, lines 59-61) (claims **13-16**).

Denning teaches a cell that carries a homozygous knock out for the alpha,1,3 GT gene, wherein at least one allele contains an induced mutation in the alpha,1,3 GT gene (see claim 1, and column 99, lines 26-27, column 23, lines 5-16).

Denning et al, teaches an animal produced by nuclear transfer cloning using the cell which carries a homozygous knock out for the alpha,1,3 GT gene, as a nuclear donor by nuclear transfer cloning technology (see claim 1, column 99, lines 26-27), (claims **43-44, 46**).

Denning et al, teaches cells from the animal that lacks expression of functional of alpha,1,3 GT gene, for use as an in vivo or ex vivo supplement or replacement for recipient cells (column 23, lines 5-19).

Denning et al, teaches cells from the animal produced by nuclear transfer technology, wherein cells can be used for an in vivo or ex vivo supplement or replacement for recipient cells (column 23, lines 5-19) (claims **48-49**). As such Denning et al, anticipates claimed invention.

Applicants argue that the Denning reference, similar to Gustafsson merely discusses the desired production of alpha 1,3GT null pigs and, like Gustafsson, fails to teach the cells and tissue of the present invention. Applicants argue moreover, the Denning specification and examples are directed almost exclusively to sheep.

These arguments are not persuasive because Denning teachings are directed to both sheep and pigs (see throughout the document).

Applicants argue that prior to this invention, no one knew whether the disruption of both alleles of the alpha- 1,3-GT gene would be lethal or would effect porcine development or result in an altered phenotype and many experts in the field expressed serious doubts as to whether

homozygous alpha-l,3-GT knockout pigs would be viable at all, much less develop normally. Applicants argue that by providing only a prophetic example based on a technique that would not be expected to succeed, neither Denning nor Gustafsson have overcome the reasonable expectation of failure that was apparent in the art. One of skill in the art reading Denning or Gustafsson would have interpreted the specification as merely a suggestion that a homozygous a(1,3)GT null mutant produced by homologous recombination would be desirable. However, they were not taught any ways to produce a viable null mutant. Applicants argue both Denning and Gustafsson merely direct others to experiment using complex techniques without providing any guidance that would be expected to produce success.

These arguments are not persuasive because claimed invention is not limited to the production of α1,3-Galactosyltransferase-Deficient Pigs, by nuclear transfer, wherein one allele of the gene is mutated at exon 9 by targeted mutation and the second allele is mutated by either natural mutation or is induced in the embryo as cited in the 2003 science paper.

Claims 1-8, 13, 17-18, 43, 48, 60, 62 are rejected under 35 U.S.C. 102(e) as being anticipated by Hawley et al, (US 2006/0242722 A1).

Hawley et al, teach the production of piglets using cell clones lacking wild-type α1,3-Galactosyltransferase (GGTA1), wherein the expression of the α1,3-Galactosyltransferase activity in the GGTA1 null animals was negative [0075] [0076]. Hawley teaches the exact nature of the GGTA1 mutation is not determined [0079].

Hawley teaches organs from said pigs comprising heart, liver, kidney, pancreas (p 9, 2nd column, claim 29) (claims 1-7).

Hawley teaches cells from said pigs comprising of fibroblasts (p 9, claim 34) (claims 13, 48).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims **1-18, 51-59, 61, 63-64** are rejected under 35 U.S.C. 103(a) as being unpatentable over **Lai et al,** (Science, 295: 1089-1092, February 2002) in view of **Straham et al,** (Frontiers in Bioscience, 1, e34-41, 1996).

Lai teaches the production of four live pigs in which one allele of the α-1,3-galactosyltransferase locus has been knocked out by nuclear transfer cloning, wherein clonal fetal fibroblasts cell lines were used as nuclear donors for embryos reconstructed for enucleated pigs (abstract and thru ought the document). Lai teaches the knock out of a α1,3-Galactosyltransferase (GGTA1) locus of exon 9 (figure 1). Lai teaches the next step will be to create α1,3-Galactosyltransferase (GGTA1)-null (honmozygous knock out pigs) by breeding from their reported produced pigs (p 1092, 1st column, 2nd paragraph). Lai have suggested the availability of galactosyltransferse-null pigs will allow a clearer evaluation of approaches currently in development aimed at overcoming potential delayed and chronic rejection mechanisms in porcine xenotransplantation (p 1092, 1st column, 2nd paragraph). Lai differs from the claimed invention by not teaching the breeding of the GGTA1 heterozygous pigs producing, homozygous pigs and cells, tissues or organs obtained from GGTA1 homozygous pigs for use as an ex vivo or in vivo supplement or replacement for recipient cell, tissues, or organs.

However, at the time the claimed invention was made, Strahan et al teach the α(1,3) galactosyltransferase epitope is the major target for human anti-pig natural antibodies leading to the events that precipitate the hyperacute rejection (p 38, 2nd column, 1st paragraph). Strahan teaches attempts are being made to produce transgenic pigs with reduced levels of expression of the α(1,3) galactosyltransferase epitope (p 37, 2nd column, 1st paragraph).
As such, Strahan et al provide sufficient motivation for one of ordinary skill in the art to breed the heterozygous α(1,3) galactosyltransferase pigs produced by Lai in order to obtain homozygous pigs with no expression of the α(1,3) galactosyltransferase.

Accordingly, in view of the teachings of Strahan et al, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to breed the heterozygous knockout pigs of Lai with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a breeding of said pigs particularly since Strahan teaches the need for the α(1,3) galactosyltransferase null epitope tissues, cells and organs for xenotransplantation and particularly since Lai have suggested the availability of galactosyltransferse-null pigs will allow a clearer evaluation of approaches currently in development aimed at overcoming potential delayed and chronic rejection mechanisms in porcine xenotransplantation.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims , **19, 44-46, 49-50** are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling **(a)** for making homozygous α(1,3) galactosyltransferase pigs by nuclear transfer by making porcine fetal fibroblasts homozygous for inactivated α(1,3) galactosyltransferase (GT) gene, by toxin A, wherein first allele was knocked out by conventional targeted homologous recombination-mediated disruption, but relied on selection of a natural mutation on the second allele of the porcine α(1,3) galactosyltransferase gene, wherein the mutation comprises the presence of a T-to G point mutation in an allele at the second base of exon 9 of the α(1,3) galactosyltransferase gene; and **(b)** breeding a male pig heterozygous for the alpha 1,3 GT gene with a female pig heterozygous for alpha 1,3 GT gene, wherein first allele was knocked out by conventional targeted homologous recombination-mediated disruption, but relied on selection of a natural mutation on the second allele of the porcine α(1,3) galactosyltransferase gene, wherein the mutation comprises the presence of a T-to G point mutation in an allele at the second base of exon 9 of the α(1,3) galactosyltransferase gene, does not reasonably provide enablement for methods of producing pigs by embryonic stem cell technology or producing α(1,3) galactosyltransferase gene DKO pigs by conventional nuclear transfer technology. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims **44-46, 49-50** are directed to a pig produced by nuclear transfer cloning using a cell carrying a homozygous knockout for the gal alpha-1,3-GT gene, wherein said cell is produced by a method comprising: (a) exposing a population of cells to C. difficile toxin A; (b) removing cells which are adversely affected by toxin A due to the receptor-mediated cytotoxicity

of the toxin; and (c) expanding and maintaining a cell that does not show the effects of receptor-mediated cytotoxicity as a nuclear donor.

As a first issue claims 44-46, 49-50 embrace a pig produced by embryonic cell (ES) knock out technology. However, it is well known in the knockout art that production of knockout non-human animals other than mice is undeveloped. This is because ES cell technology is presently limited to the mouse system as only mouse ES cells achieve germline transmission of a disrupted target gene (**Hochepied et al,** Stem Cells, 22: 441-447, 2004) (abstract). Given the undeveloped and unpredictable nature of ES cells it would have required undue experimentation for the skilled artisan to use embryonic stem cells other than mouse without a reasonable expectation for success.

As a second issue claims **44-46, 49-50** are directed to a pig produced by nuclear transfer cloning using a cell carrying a homozygous knockout for the gal alpha-1,3-GT gene, wherein said cell is produced by a method comprising: (a) exposing a population of **cells** to C. difficile toxin A; (b) removing cells which are adversely affected by toxin A due to the receptor-mediated cytotoxicity of the toxin; and (c) expanding and maintaining a cell that does not show the effects of receptor- mediated cytotoxicity as a nuclear donor.

The specification teaches the production of **primary porcine fetal fibroblasts** heterozygous for the alpha-l,3-GT gene (example 1); the production of primary porcine fetal fibroblasts homozygous for the alpha-l,3-GT gene (example 2); the selection with C.difficile Toxin A for primary porcine fetal fibroblasts homozygous for the alpha-l,3-GT gene (example 3); and the generation of cloned pigs using homozygous alpha 1,3 GT-Deficient fetal fibroblasts as nuclear donors (example 4). However, the specification has failed to provide guidance for the

production of any other cells other than primary porcine fetal fibroblasts homozygous for the alpha-l,3-GT gene.

In view of the state of the art in somatic NT cloning at the time, the art was still under-developed and many known or unknown barriers hamper the success. For example, (**Denning et al,** Nat Biotech 2001 June;19:559) teaches the difficulties of somatic cell targeting, "a substantial number of colonies with only targeted cells senesced before they could be prepared for nuclear transfer. The high attrition rate of targeted clonal populations suitable for nuclear transfer represents one of the major hurdles of gene targeting in primary somatic cells" (left column, page 560, emphasis added). The general state of the art was such that somatic NT cloning in pigs was and still is highly inefficient, and the underlying mechanism for such inefficiency had not been fully understood, which reflects the under-developed state of the art, and such inefficiency cannot be resolved by routine experimentation. **Yanagimachi** (Mol Cell Endocrinol 2002;187:241-8) teaches that "cloning efficiency-as determined by the proportion of live offspring developed from all oocytes that received donor cell nuclei-is low regardless of the cell type (including, embryonic stem cells) and animal species used. In all animals except of japanese black beef cattle, the vast majority of cloned embryos perish before reaching full term" (abstract), and "thus far, cloned offspring that survived birth and reached adulthood were the exception rather than the rule (page 243, left column, emphasis added). Yanagimachi goes on to teach, "this low efficiency of cloning seems to be due largely to faulty epigenetic reprogramming of donor cell nuclei after transfer into recipient oocytes. Cloned embryos with major epigenetic errors die before or soon after implantation" (abstract). Wells et al (Trends Biotechnol 2003:21:428-32) teach that the continuous loss of clones throughout pregnancy and high mortality during the perinatal period raise serious animal welfare concerns and these losses can mostly be attributed to faulty epigenetic reprogramming of the donor cell genome,

resulting in major dysregulation of gene expression (paragraph bridging left & right column in page 1). In light of the state of the prior- and post-filing art supra, the outcome of instant working examples does not appear to be just a random incidence because the skilled artisans teach that fetuses not surviving to term often reflect the real difficulty and major challenge in somatic cell NT cloning.

Producing a somatic pig cell homozygous for inactivation of α1,3GT gene in culture other than the disclosed primary fetal fibroblasts has not been evidenced.

The state of the art pertaining to making homozygous knockout cells, difficulties exist in both gene targeting and targeted cell selection processes. For example, somatic cells prepared for nuclear transfer are difficult to sustain in vitro due to their innate viability. **Denning et al** (Nat Biotech 2001;19:559-562) teach, "a substantial number of colonies with only targeted cells senesced before they could be prepared for nuclear transfer. The high attrition rate of targeted clonal populations suitable for nuclear transfer represents one of the major hurdles of gene targeting in primary somatic cells" (left column, page 560).

Phelps et al, (Science, 299: 411-414, 2003) notes making pig cells homozygous for α1,3GT inactivation "Although our intent was to knock out the second allele of the α1,3GT gene by homologous recombination, this did not occur" (lines 1-4, right column, page 413, emphasis added). The statement confirms the difficulty and unpredictability of double knock-out a gene in a somatic cell. The specification as well as in the publication, Phelps et al employed a new method for selecting α1,3GT-double negative cells, i.e. a "toxin A selection" method, which is critical in achieving the goal of obtaining a porcine cell homozygous for inactivated α1,3GT

gene. Phelps et al teach, "The fact that one normal-sized allele was observed (instead of two shorter knockout alleles) indicated that knockout of the second α1,3GT allele was due to mechanisms other than targeted homologous recombination-mediated disruption, promoter dysfunction, or mRNA missplicing and instability" (mid- and right column, page 412). Apparently, Phelps et al failed to knockout the second allele of the porcine α1,3GT gene in a somatic cell using instantly claimed, conventional targeted homologous recombination-mediated disruption, but relied on selection of a natural mutation on the second allele of the porcine α1,3GT gene. Phelps et al went on to teach "because we used this powerful selection method, which allows us to isolate any event that results in loss of α1,3GT activity, we discovered a mutation in the second allele of the α1,3GT gene. Had we used standard selection methods with puromycin or hygromycin, we would not have found the mutation" (right column, page 413, emphasis added). The Phelps publication confirms the difficulty of double knockout a gene in a somatic cell, which has not been resolved using routine experimentation even long after instant priority date.

Kuroiwa et al (Nat Genetics 2004;36:775-80), gives a clear view regarding the state of the art in gene targeting of somatic cells in culture for NT cloning. Kuroiwa et al teach, "Gene targeting in somatic cells versus embryonic stem cells is a challenge; consequently, there are few reported successes and none include the targeting of transcriptionally silent genes or double targeting to produce homozygotes" (see e.g. abstract, emphasis added). Kuroiwa et al teach breeding to homozygosity is severely impeded in species that have a long generation interval, such as cows, sheep and **pigs,** further they are negatively impacted by the consequences of inbreeding. Kuroiwa et al particularly mentioned "innovative" approaches to obtain homozygous α (1,3)GT knockout pigs as reported by Phelps et al, but pointed out "unfortunately, these approaches are neither useful for silent genes nor widely applicable for

active genes" (right column, page 775). The success of Kuroiwa et al was brought about by another innovative approach, i.e. sequential application of gene targeting by homologous recombination and rejuvenation of cell lines by cloned fetuses. Such approach was developed long after the instant filing date, and the specification fails to teach such a method, thus, the reference does not support the enablement of instant claimed invention. To the contrary, it confirms that it requires further development and undue experimentation to enable the instantly claimed invention.

Shi et al, (Differentiation, 671(2): 91-113, 2003) notes somatic cloning is an inefficient and unpredictable process, and a plethora of anomalies have been described in cloned embryos, fetuses and offsprings due to incomplete or inappropriate epigenetic reprogramming of donor nuclei is likely to be the primary cause of failures in nuclear transfer from different species (abstract). **Dinnyes et al,** (Cloning and Stem Cells, 4(1): 81-90, 2002) notes that there are several species where attempts to somatic cell cloning have been unsuccessful and the reasons for this lack of success varies between species (p 82, 2nd column 2nd paragraph). **Dinnyes et al,** further notes the effect of genetic background on nuclear transfer, wherein further studies are needed to identify which genetic combinations of donor cells and recipient cytoplasts are the most successful and whether the extent of the problem is similar in all species (p 82, 2nd column, last paragraph bridge p 83, 1st column, 1st paragraph).

The specification fails to teach how to overcome the difficulties as taught by Denning et al, it fails to teach the innovative methods as taught by Phelps et al and Kuroiwa et al, thus, the success of Phelps et al and Kuroiwa et al do not support the enablement for making pig cells homozygous for α1,3GT inactivation other than the disclosed primary fetal fibroblasts for production of homozygous alpha, 1, 3 GT pigs at the time the application was filed. The specification fails to teach how to overcome the art known hurdles in knocking out the second

allele of any porcine cell in culture other than the disclosed primary fibroblasts, it fails to teach the innovative methods found in the post-filing art, and thus it fails to provide an enabling disclosure for instantly claimed invention.

In conclusion, knocking out the second allele of the porcine α1,3GT gene via homozygous recombination-mediated disruption in a heterozygous porcine somatic cell was not routine in the art, had not reduced to practice, and had not been achieved in pigs at the time of instant filing, and it would have required undue experimentation for the skilled intending to practice the claimed invention.

Claims 49-50 are drawn to obtaining cells or tissues from said pigs for use as an in vivo or ex vivo supplement or replacement for recipient cells, tissues, or organs. It is well known in the art that Gal(1,3)Gal determinant is the major antigen responsible for hyperacute rejection response in xenotransplantation. Solving the problem of hyperacute rejection does not make xenotransplantation feasible. The specification contemplates that cells, tissues or organs derived from such porcine animals would be useful for xenotransplantation (specification p 9, lines 15-16).

The art teaches that it would not render the xenotransplantation feasible because the tissue or organ devoid of antibody detectable Gal(1,3)Gal determinants will still face vigorous host rejection response when used in xenotransplantation. This is because Gal(1,3)Gal determinant is only one of the many factors that trigger xenograft rejection responses. **Platt et al** (Nat Biotech 2002 Mar; 20(3)231-2) clearly teach, "Unfortunately, solving the problem of hyperacute rejection does not make xenotransplantation feasible, but rather reveals a more vexing problem called acute vascular rejection. Acute vascular rejection, like hyperacute rejection, is triggered by anti-donor antibodies; however, in contrast to hyperacute rejection, these antibodies are not directed exclusively against α1,3Gal, and the involvement of the

complement system is far more subtle" (Emphasis added). **Sharma et al,** (Transplantation, 75: 430-436, 2003) notes that fetal-pig fibroblasts homozygous for the knockout of the α1,3GT gene appear to express low level but detectable levels of the gal antigen and two lines of evidence suggest that low levels of antigen expression could still be problematic for xenotransplantation (p 435, 1st column, 2nd paragraph). In view of such knowledge, the claimed cells, tissues or organs do not appear to be enabled in the absence of evidence to the contrary.

Therefore, in view of the quantity of experimentation necessary to determine the parameters listed above for the cloning of a pig homozygous for the knockout of the α1,3GT gene by conventional nuclear transfer technology or by breeding α1,3GT heterozygous pigs, the lack of direction or guidance provided by the specification for the cloning of a pig homozygous for the knockout of the α1,3GT gene by conventional nuclear transfer technology or by breeding α1,3GT heterozygous pigs, the absence of working examples that correlate to the production of said pigs, the unpredictable state of the art with respect to production of said pigs, and the breadth of the claims directed to any somatic cell homozygous for the α1,3GT gene knocked, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention other than the disclosed pigs produced by the disclosed primary fibroblast selection and by breeding of the disclosed α1,3GT heterozygous pigs.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 47 rejection for insufficient antecedent basis for this limitation in the claim and its dependency from claim 42 which is directed to a cell is **withdrawn.**

Claims 20 and 21 are free of art.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

Magdalene K. Sgagias, Ph.D.
Art Unit 1632

/Anne-Marie Falk/
Anne-Marie Falk, Ph.D.
Primary Examiner, Art Unit 1632